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and additional genomic sequences may be necessary for appropriate Hoxa13-like expression.

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Hox11 genes interact with Eya1 and Pax2 to activate Six2 and Gdnf expression during metanephric kidney induction

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The Hox complex of genes is critical to many developmental processes, but few pathways by which Hox transcription factors regulate downstream genes have been defined. Indeed, very few downstream targets of Hox genes have been identified. By removing all six functional alleles of the Hox11 paralogous group genes, we generated animals which have no kidneys. The metanephric blastema forms early in development, but no ureteric bud induction occurs. This phenotype occurs with 100% penetrance and is due to loss of the expression of Six2 as well as Gdnf, the inducing ligand for ureteric budding. We have shown that Hox11, Eya1 and Pax2 proteins physically interact and synergistically regulate the expression of Six2 and Gdnf, identifying two new potential downstream targets of Hox genes. Furthermore, a single site has been identified in the Six2 promoter region that binds the Hox11–Eya1–Pax2 complex and confers Hox–Eya–Pax-mediated transcriptional activity. Preliminary molecular and genetic studies demonstrate that interaction between other Hox paralogous groups and the conserved Pax–Eya–Six network exists and suggest that Hox interaction and coregulation with the Pax–Eya–Six regulatory network may represent a conserved developmental mechanism.

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Molecular analysis of a polydactylous chicken

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Spontaneous mutations in vertebrate organisms have been successfully used to elucidate information about limb patterning. We analyzed a spontaneous mutant of the species *G. gallus* commonly referred to as "Dorkings" in an attempt to identify the molecular basis for the formation of a polarized extra hindlimb digit. Two signaling centers have been implicated in producing polarized ectopic digits: the apical ectodermal ridge (AER) and the zone of polarizing activity (ZPA). The AER forms through the condensation of ectodermal cells located on the distal edge of the limb bud. The ZPA is defined as distal, posterior mesenchymal tissue that expresses the secreted protein sonic hedgehog (Shh). In Dorking embryos, we have found that the AER markers fibroblast growth factors 4 and 8 (Fgf4 and Fgf8) were ectopically expressed, Shh was not ectopically expressed, and the Shh target gene Patched 1 (Ptc1) was also not ectopically expressed in Dorking embryos. These data suggest that in Dorking chickens expanded Fgf expression does not cause ectopic Shh signaling but may directly result in polydactyly. Currently, we are validating results by real time PCR and testing the role ectopic Fgf expression plays in ectopic digit formation by recombining mutant ectoderm with wild type mesoderm in organ culture experiments.

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Hox-dependent regulation of *Rhomboid* during chordotonal organ development

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Each segment of a *Drosophila* embryo develops a stereotypic pattern of sensory organs. The correct patterning of sensory organs during development depends on inputs of proneural genes and anterior–posterior positional cues. One type of sensory organ, the chordotonal organ, consists of from 1 to 80 closely associated sensory structures called scolopodia. Each scolopodium arises from a single sensory organ precursor (SOP) cell that divides to form four cell types: a neuron, a scolopale (glial) cell, a cap cell, and a ligament cell. Chordotonal organs exhibit Hox-dependent differences in abdominal versus thoracic segments. One set of serially homologous chordotonal organs, for example, differ in the number of scolopodia (3 in thorax versus 5 in abdomen) and their position (dorsal in the thorax versus lateral in the abdomen). Here, we identify an enhancer of *Rhomboid* (Rho654), a gene important in chordotonal organ development, which is sufficient to drive expression in one neuronal precursor cell of abdominal chordotonal organs. We show by transgenic fly reporter assays that expression of Rho654 in vivo is hox-factor-dependent and dependent on input from neural factors. We also show by DNA binding assays that complex formation can occur between hox factors and cofactors on sequences found in Rho654. Thus, we provide direct evidence of regulation of Rhomboid by Hox factors on an enhancer specific to one SOP in abdominal chordotonal organs.

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The role of Tbx20 in endocardial cushion cell proliferation and extracellular matrix remodeling during heart valve development

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In the US, congenital heart defects (CHD) are among the most common birth defects, occurring in approximately 1/100 live births. The majority of CHDs involve abnormal valvulo-septal development, however, the molecular mechanisms underlying valve development remain relatively unknown. The T-box family of transcription factors is known to be involved in several aspects of heart development including cardiac lineage determination and chamber specification. Recent studies suggest that Tbx20 is a likely candidate for regulating valve development. In murine embryos, tbx20 is expressed in the myocardium where it is required for proliferation. Tbx20 is also expressed in the endocardial cushions (EC) of the atrioventricular canal and remodeling valves, however, its function is unknown. Upstream regulators and downstream targets of Tbx20 were examined in avian EC cells in order to elucidate the function of Tbx20 in developing valves. Previous studies have shown that Bmp2 is necessary for EC formation. We show that, in EC cells, tbx20 is induced by the Bmp2 pathway. In addition, Tbx20 gain and loss of function studies demonstrate that Tbx20 increases the expression of extracellular matrix genes including aggrecan and versican and decreases the expression of matrix metalloproteinases including mmp9 and mmp13. Additionally, Tbx20 promotes proliferation in EC cells. Taken together, these data support a role for Tbx20 in proliferation and extracellular matrix remodeling during valve development.

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Snail family genes are required for left–right asymmetry determination but not neural crest formation in mice

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Members of the *Snail* gene family regulate epithelial–mesenchymal transitions (EMT) by repressing the transcription of components of cell adhesion complexes. EMT occurs several times during development, including gastrulation and delamination of neural crest cells from the dorsal neural tube. We reported previously that *Snail (Snai1)* null mouse embryos displayed gastrulation defects, which die by 7–8 days of gestation, precluding examination of the role of *Snai1* in later developmental events. Using a conditional allele and the Meox2-cre line, we are able to rescue the *Snai1* null phenotype through 9.5 days of gestation. Here, we show that, contrary to observations in frog and avian embryos, *Snai1* and *Slug (Snai2)* are not required for formation and delamination of the neural crest in mice. In both $Snai1^{flox/-}$; Meox2-Cre *(Snai1-cko)* and *Snai1-cko*;$Slug^{-/-}$ compound mutant embryos, neural crest cells form, delaminate and appear to migrate properly into the branchial arches. However, in addition to gastrulation defects, the *Snai1-cko* embryos exhibit multiple laterality defects, including randomization of the direction of heart looping and embryonic turning. The left determinant genes *Nodal*, *Lefty2* and *Pitx2* display bilateral expression patterns that are particularly prominent in the posterior region, overlapping the normal *Snai1* expression domain. These changes are independent of gross structural defects at the midline and in the node. Our findings suggest that *Snai1* is not required for delamination of the neural crest in mice but plays a critical role in the determination of left–right asymmetry.

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Foxd3 is required for neural crest development

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The neural crest (NC) cells disperse from the dorsal surface of the neural tube and migrate extensively through the embryo, giving rise to a wide variety of differentiated cell types. Foxd3, a member of winged helix transcription factor family, is expressed early in the preimplantation and gastrulating embryo, later in the premigratory and migrating neural crest and some differentiated NC derivatives. Previous studies showed that Foxd3 is sufficient to direct neural cells towards the NC lineage and is required for maintenance of two disparate stem cell types from the early mouse embryo: embryonic stem cells and trophoblast stem cells. The null mutant embryos die around 6.5 dpc, before the NC is specified. Here, we conditionally inactivated the *Foxd3* gene specifically in the NC using the Cre/LoxP recombinase system. $Foxd3^{flox/-}$;*Wnt1-cre* embryos die perinatally with profound deficiencies in cranial, trunk, vagal and sacral neural crest, including severe craniofacial cleft and missing bones of the head, hypoplastic cranial nerves and pharyngeal arches, defects of peripheral nervous system and complete absence of enteric nervous system. However, the heart and its outflow tract appear grossly normal. $Foxd3^{flox/-}$;*Wnt1-cre* embryos have apoptotic cells located at the dorsalmost region of the neural tube where wild type embryos do not, and cell proliferation is unchanged. Our results establish a requirement for Foxd3 in NC development. Experiments are in progress to understand the interaction of Foxd3 with signal transduction networks and transcription factors controlling migration and differentiation of NC in vivo and maintenance of NC stem cells in vitro.

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Early specification of neural crest and the role of Pax7 on its development

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Neural crest cells are a dynamic migratory stem cell population that differentiates into a plethora of derivatives,